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**From:** Kerr, Janet  
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Please order the following references:

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Thanks,  
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## Embryonic Stem Cells

### TEC-1 CHARACTERISATION OF PORCINE EMBRYONIC CELLS FROM DAY 11 EMBRYONIC DISCS CULTURED IN SERUM-FREE MEDIUM

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Day-11 porcine embryonic discs express cell phenotype markers typical of undifferentiated murine embryonic stem cells (ESCs): they are positive for the stage specific marker TEC-1/SSEA-1 (a potential marker of totipotency), but are negative for cytokeratin 8/18. However, culture of such discs in serum-containing medium induces partial differentiation whereby expression of TEC-1 disappears; whereas, cytokeratin expression becomes evident (Wianny et al., 1995, Soc. Franç. Étud. Fert. 34<sup>e</sup> Réun., Montpellier, Abst. C2). This differentiation may be induced by the growth factors and retinoids contained in serum. Hence, a serum-free medium was developed to re-examine TEC-1 expression patterns in primary cultures of porcine embryonic disc cells derived from day-11 blastocysts.

Embryonic discs were microsurgically dissected free of trophectoderm and endoderm and cultured on a fibronectin substrate ( $1 \mu\text{g}/\text{cm}^2$ ) in DMEM/F12 medium supplemented with  $10 \mu\text{g}/\text{ml}$  insulin,  $5.5 \mu\text{g}/\text{ml}$  transferrin,  $6.7 \text{ ng}/\text{ml}$  selenium,  $0.1 \text{ mM}$   $\beta$ -mercaptoethanol and either 10 or 100 ng/ml human leukaemia inhibitory factor (LIF): addition of 100 ng/ml LIF to this medium permitted 24/44 (54.5%) of attached embryonic discs to continue to expand after the third day in culture; whereas, 0/26 (0%) of discs cultured in only 10 ng/ml LIF survived, as all detached from the substrate at this time. Colonies cultured in 100 ng/ml LIF were stained for TEC-1 from the 3-5<sup>th</sup> day of culture. Compared to typical colonies cultured in the presence of serum, remarkably different TEC-1 expression patterns and cellular morphologies were observed in serum-free medium. Three major cell phenotypes could be identified: a) cells possessing a high nucleo-cytoplasmic ratio that appeared to be directly derived from the embryonic disc that were variably stained for TEC-1. Such cells appeared to be differentiating into two other cell types: b) epithelial-like polygonal cells staining both positively and negatively for TEC-1, so as to produce a mosaic-type staining pattern, c) cells of low nucleo-cytoplasmic ratio that formed rapidly expanding populations that were negative for TEC-1. TEC-1 positive cell populations accounted for  $32.3 \pm 31\%$  (mean  $\pm$  sd) of the total area of the colonies ( $n=16$ ). Individual addition of other growth factors (10 ng/ml basic fibroblastic growth factor ( $n=8$  colonies), 100 ng/ml epidermal growth factor ( $n=18$ ) or 100 ng/ml insulin-like growth factor-II ( $n=10$ )) together with LIF (100 ng/ml) did not increase the percentage area represented by the TEC-1 positive cell populations ( $24.9 \pm 33$ ,  $31.3 \pm 30$ , and  $29.4 \pm 28\%$ , respectively,  $P > 0.05$ ).

These results indicate: i) high concentrations of LIF are essential to maintain colonies in serum-free medium, ii) removal of serum (or specific serum components) permits continued expression of TEC-1 which, if analogous to murine ESCs, suggests a maintenance of totipotency in embryonic disc-derived cultured cells. Further examination of the growth factor requirements that can specifically expand these TEC-1 positive populations is required.

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